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(54) Title: PEPTIDE-MEDIATED MODULATION OF T-CELL RECOGNITION AS A MEANS OF AFFECTING IMMUNE RESPONSES

(57) Abstract

A potentially large group of endogenous peptides is involved in mediating MHC-restricted T-cell recognition of antigens, and that recognition can be modulated by means of peptides that replace such endogenous peptides at the binding site on the class II molecule which interacts with the T-cell receptor. The replacing peptides can thus be used to regulate mammalian immune responses by influencing the recognition of an alloantigen or an autoantigen by T cells, for example, in the context of treating an immune disorder, overcoming allograft rejection or affecting an allergenic response.

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PEPTIDE-MEDIATED MODULATION OF T-CELL RECOGNITION AS A MEANS OF AFFECTING IMMUNE RESPONSES

Background of the Invention

The responsiveness of the cellular immune system is determined by an array of highly polymorphic, so-called "class II" molecules encoded by genes comprising the major histocompatibility complex (MHC) expressed in the membranes of macrophages and other antigen-presenting cells (APCs), B-lymphocytes (B cells), monocytes and some T-lymphocytes (T cells).

Class II MHC molecules are transmembrane glycoproteins of APCs that bind peptide fragments of foreign proteins so that they can be presented to $CD4^+$ (helper) T cells. Receptors on the surface of a $CD4^+$ T cell thus form ternary complexes with the class II molecule and the peptide fragment. This leads, in turn, to the activation of the T cells and the subsequent development of an immune response.

A given T cell is specific for a peptide and is MHC-restricted as well, in the sense that the T cell recognizes the peptide only when the latter is bound to a particular class II molecule. Class II MHC molecules are therefore directly involved in mediating the immune response of mammals, for example, in the context of

transplant rejection (since it is the binding of an antigen of the allograft by a class II molecule that can initiate such rejection) and of reactions to an environmental allergen. Class II molecules are also thought to play a role in the etiology of "autoimmune" diseases, like rheumatoid arthritis, ankylosing spondylitis, pemphigus vulgaris, insulin-dependent diabetes mellitus and multiple sclerosis, in which the body's immune system attacks self protein antigens.

Mixed results have attended past efforts to develop ways of alleviating the respective effects of transplant rejection and various autoimmune disorders. Thus, the use of different immunity-suppressing agents, such as cyclosporin, the corticosteroids and other cytotoxic drugs, suffers the drawback of nonspecificity, i.e., the agent is typically toxic to other systems and may place a patient at risk of cancer and other disorders.

Summary of the Invention

It is therefore an object of the present invention to provide a means for regulating mammalian immunity so as to influence the recognition of an alloantigen, an allergen, an autoantigen by T cells, e.g., in the context of an immune disorder or rejection of a transplant.

It is also an object of the present invention to provide a method for treating autoimmune diseases that is specific and, hence, does not impair immune responses to other antigens.

It is yet another object of the present invention to provide a method of mitigating or preventing the rejection of a transplant, which method does not depend on a nonspecific suppression of the body's immune system.

In accomplishing the foregoing objects, there has been provided, in accordance with one aspect of the present invention, a method for affecting immune

responses in a mammalian subject, comprising the step of administering to the subject a replacing peptide capable of occupying an MHC binding site in lieu of an endogenous peptide, wherein the replacing peptide is administered to the subject in an amount sufficient to modulate T-cell recognition of an MHC class II molecule:endogenous peptide complex. In one embodiment of the present invention, the replacing peptide is a derivative of influenza virus hemagglutinin protein.

There has also been provided, in accordance with another aspect of the present invention, a pharmaceutical composition for affecting immune responses in a mammalian subject, comprising an amount of a replacing peptide capable of occupying an MHC binding site in lieu of an endogenous peptide, wherein the amount of the replacing peptide is effective to modulate T-cell recognition of an MHC class II molecule:endogenous peptide complex. Likewise provided is the use of a therapeutic amount of such a replacing peptide in the manufacture of a medicament for use in a method for affecting immune responses in a mammalian subject, that method being characterized by an administration to the subject of an amount of the replacing peptide sufficient to modulate T-cell recognition of an MHC class II molecule:endogenous peptide complex.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

Figure 1 is a graphical representation of peptide-mediated modulation of alloreactive T-cell clones, pursuant to the present invention. As described in greater detail below, HLA-DR1-specific, alloreactive T-cell clones were stimulated with varying concentrations of irradiated, $DR1^+$ lymphoblastoid "stimulator" cells that had been pre-incubated with a synthetic peptide derived from influenza virus hemagglutinin protein. Negative controls consisted of lymphoblastoid cells alone and clone alone in tissue culture medium supplemented with 10% pooled human plasma. After a 48-hour incubation period, each microwell was pulsed overnight with 1.0 μCi ^{3}HTdR (specific activity 6.7 Ci/mM). Radiolabel incorporation was measured by liquid scintillation spectroscopy and expressed as the mean counts per minute (CPM) of triplicate cultures.

Figure 2 is a series of bar graphs illustrating the relative capacity of a number of synthetic peptides, in accordance with the present invention, to inhibit (2a) and enhance (2b) T-cell proliferation responsive to allostimulation.

Detailed Description of Preferred Embodiments

It has been discovered that a potentially large group of endogenous peptides is involved in mediating T-cell recognition of antigens and, in addition, that such recognition can be modulated by the use of a peptide that replaces endogenous peptide at the binding site on the class II molecule that interacts with the T-cell receptor. More specifically, peptides can be identified that replace endogenous peptides selectively at the class II binding site and, thereby, counteract pathological immune responses associated, for example, with autoimmune disease, as discussed by Todd et al, Science 240: 1003-

09 (1988), the contents of which are hereby incorporated by reference; the rejection of an allograft; "graft-versus-host" disease, as occurs when foreign T cells respond to antigens of a patient receiving the T cells in bone marrow transplant; and allergic responses to environmental allergens. Matching of the appropriate replacing peptide to the desired counteraction of a immune response can be accomplished empirically, via judicious selection of peptide or the modification of residues of a known peptide.

Class II molecules consist of two non-covalently linked peptide chains (α and β) which traverse the plasma membrane, each chain having two domains on the outside of the cell. Both chains can be polymorphic, although there is more structural variation in the β chains. In any event, the variation in class II molecules between individuals can mean that a given replacing peptide will be effective in counteracting a particular immune response for some individuals but not others. To accommodate this exigency, different combinations of replacing peptides can be identified empirically on the basis of relative abilities to modulate responses restricted by different class II allelic products.

The precise identity of any endogenous peptide is unknown at present, as is the amount of variation among the class of such peptides. Pursuant to the present invention, however, knowledge concerning the nature of the peptide involved in the class II molecule-endogenous peptide complex is unnecessary; rather, one need only employ a replacing peptide that is determined empirically to bind <u>in vivo</u> for site on the class II molecule which otherwise binds the endogenous peptide.

Among the peptides suitably used as replacing peptides in accordance with the present invention are those that comprise the amino-acid sequence Cys-Pro-Lys-

Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly, which represents the residues 306 through 320 of influenza virus hemagglutinin (HA) protein. It has been found that synthetic polypeptides containing this sequence and, optionally, additional amino-acid residues can modulate immune responses in the manner described above and, hence, are appropriate candidates for replacing peptides in a clinical context. The same is true of polypeptides containing a variant of the aforementioned sequence obtained, for example, by deleting one or two residues from at least one end of the sequence. Such a variant is one that includes the sequence Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr, as are sequences derived therefrom via one-, two- or three-residue substitutions in the variant sequence.

According to the present invention, it is important for the replacing peptide to be present in a therapeutically-effective concentration <u>in vivo</u>. Generally, such concentrations will be on the order of 100 micromolar in magnitude, but a suitable concentration for a given treatment will be determined in practice on a case-by-case basis.

To this end, the replacing peptide can administered systemically, for example, via intravenous or intraperitoneal injection in physiologic saline or other physiologically compatible carrier, or (in the context of a transplant) by bringing the foreign tissue into infiltrating contact with a physiologically compatible solution containing the replacing peptide. For treatment of an autoimmune disorder, administration may be optimally accomplished by localized injection at the site where symptoms of the disorder are manifest, e.g., at a joint affected by inflammation associated with rheumatoid arthritis.

The present invention is further described below by reference to the following illustrative examples.

Example 1. Modulation of T-cell recognition with an HLA-DR1-restricted peptide representing a portion of influenza virus hemagglutinin (HA) protein.

GENERATION OF ALLOREACTIVE T-CELL CLONES: To generate DR1-specific, alloreactive T-lymphocyte clones (TLCs), peripheral blood lymphocytes (PBLs) were isolated from HLA-DR1-negative, normal individuals by density gradient centrifugation over Ficoll-Hypaque and stimulated with allogeneic PBLs that had been irradiated (30 Gy) PBLs selected as stimulators were HLA-DR-serotyped by direct complement-mediated cytotoxicity after an enriching for B cells on solid-phase antihuman immunoglobulin, according to Grier et al, Tissue Antigens 10: 236-37 (1977). Priming combinations included cells bearing HLA-DR1 through DRw8 antigens, and was effected against alloantigens associated with two complete haplotypes.

Responder and stimulator cells were each adjusted to 1 x 10^6/ml in medium containing 10% human plasma, combined 1:1 in 2-ml aliquots and placed into sterile, round-bottom tests tubes. After six days of incubation at 37° in 5% CO_2 air, viable cells were harvested by pooling the cells and layering over Ficoll-Hypaque. Cells were centrifuged for thirty minutes at 1000 x g, and viable cells were collected from the gradient interface. Cells were thereafter resuspended in 1 ml of medium containing 10% human plasma and 20% interleukin-2 (T-cell growth factor or TCGF), the latter obtained from phytohemagglutinin-activated, normal PBCs as described by Rosen-Bronson et al, Immunogenetics 23: 368-78 (1986).

The primed lymphoblasts were cloned by limiting dilution, in the presence of TCGF and a fresh alloantigenic challenge, according to the technique of

Eckels and Hartzman, Hum.Immunol. 3: 337-43 (1981). Thus, primed cells were diluted to 18 cells/ml in medium containing 10% human plasma, 20% TCGF, and the original, irradiated stimulator PBLs at 6 x 10^5/ml. These cell mixtures were plated in 16-μl aliquots in sterile, 60-well Microtest II trays (Falcon). Trays were placed in humidified chambers and incubated at 37°C in 5% CO_2 air for seven days. After one week, the trays were examined for wells containing proliferating cells.

Positive wells were transferred to 96-well U-bottom trays containing 1 x 10^5/well irradiated stimulator cells in 0.1 ml medium plus 10% human plasma. After three days in the absence of TCGF, 0.1 ml of complete medium supplemented with 10% human plasma plus 20% TCGF was added to each well. After a total of seven days in 96-well trays, proliferating clones were transferred in 24-well culture trays containing 1 ml/well of medium (10% human plasma, 20% TCGF) and 1 x 10^6/ml irradiated stimulators (30 Gy).

Clones generated in this manner were maintained on a regular schedule of 20% TCGF on day 3, followed by irradiated stimulator (feeder) cells plus 20% TCFG on day 7. Clone concentrations were kept at 2-4 x 10^5/ml, and feeder cells were added at a final concentration of 1 x 10^6/ml. Clones were transferred to progressively larger containers when necessary. The resulting TLCs were frozen at -180°C before being thawed and screened in proliferation assays for their specificities on panels of allogeneic PBLs or lymphoblastoid B-cell lines (LCLs).

Three series of alloreactive TLCs were derived: Series 61 (DR2;DRw13 anti-DR1), Series 62 (DR2;DRw14 anti-DR1;DR2) and Series 63 (DR2;DRw13 anti-DR1;DR2). Thus, DR1-associated allodeterminants were primarily recognized, since DQ and DP antigens were shared.

MODULATION OF ALLOREACTIVE TLC PROLIFERATION BY REPLACING PEPTIDE: The proliferative response of the alloreactive TLCs was determined in the presence of a synthetic replacing peptide known to be presented by $DR1^+$ APCs. See Eckels et al, Immunogenetics 19: 409-23 (1984); Lamb et al, Nature 300: 66-69 (1982). The peptide consists of amino-acid residues 306 through 320 of HA protein
("$HA_{306-320}$"), that is:
Cys-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly (I).

More specifically, the proliferative response of the TLCs (1×10^4/well) were determined while concentrations of $HA_{306-320}$ were held constant at 30 or 100 μg/ml, and the number of LCL stimulator cells was increased (range: 2.5×10^4 to 20×10^4 cells/well). The alloreactive TLCs were added, respectively, under suboptimal stimulating conditions in which LCL cells with bound peptide would be limiting at low cell concentrations; suboptimal conditions ensured that any modulating effects of the replacing peptide would be detected.

As a control, LCLs were preincubated with a synthetic peptide representing residues 51 through 65 of ragweed antigen E (Glu-Val-Trp-Arg-Glu-Glu-Ala-Tyr-His-Leu-Ala-Asp-Ile-Lys-Asp). This peptide has also been shown to be restricted by HLA-DR1. See Rothbard et al, Cell 52: 515-23 (1988).

To assay the TLC proliferation response, LCL stimulator cells were irradiated (10^4 rads) and resuspended at the above-mentioned concentrations in RPMI 1640 tissue culture medium supplemented with 10% human plasma, 2 mM glutamine, 25 mM HEPES,
50 μg gentamicin per ml, 100 μg of streptomycin per ml, 100 international units (IUs) of penicillin per ml, and 24 IUs of sodium heparin per ml. Whenever peptide was

employed, it was added, at the concentrations indicated above, two to twenty-four hours prior to the addition of TLCs.

Responder TLCs were plated, at 1 x 10^4 cells per well, in supplemented medium. Triplicate cultures (200 μl) were incubated for forty-eight hours at 37°C in 5% CO_2/air and then pulse overnight with 1.0 μCi titrated thymidine. As correlated with incorporation of radiolabel, proliferation was measured by liquid scintillation spectroscopy and expressed as the mean counts per minute of triplicate cultures (± SEM).

No effect was detected when LCLs were preincubated with the control peptide derived from antigen E of ragweed. By contrast, a majority (62%) of the human alloreactive T-cell clones was inhibited by the addition of the replacing peptide. As shown in Figure 1, three patterns of alloreactivity modulation were observed with the HA-derived replacing peptide. The first pattern, exemplified by TLC AL63.14 (Figure 1a), included five of eight T-cell clones wherein recognition of alloantigen was inhibited to varying degrees by increasing concentrations of the replacing peptide. At an LCL concentration of 1 x 10^4 cells/well, inhibition of clonal proliferation in response to allostimulation ranged from 38% to 86% at 100 μg of replacing peptide per milliliter.

One T-cell clone, AL63.65, was enhanced two- to six-fold in its ability to proliferate in response to allostimulation (Figure 1b). Notably, AL63.65 was also found not to be stimulated by the HA-derived peptide and autologous APCs; in addition, the peptide bound to the original priming $DR1^+$ LCL induced the same enhancement. A contrasting pattern was observed with the clones AL61.102 (Figure 1c) and AL63.75 (Figure 1d), the alloreactivity of which was not affected by the replacing peptide.

To show that the modulating effect of the replacing, HA-derivative peptide was due to peptide interaction with the DR1 molecule, rather than to some secondary effect on class II expression, paraformaldehyde was used to fix stimulator cells, respectively, before and after addition of the peptide in an assay involving clone AL63.65. More specifically, paraformaldehyde was freshly diluted to 0.01% in sterile, normal saline and adjusted to pH7.2 with HCl. LCLs (2×10^6) were aliquoted into 12 x 75 mm sterile test tubes and pelleted at 200 x g for 10 minutes. Supernatant was aspirated, the pellet was resuspended, 1 ml of 0.01% paraformal- dehyde was added, and the resulting mixture was incubated at room temperature for 20 minutes. Unsupplemented RPMI 1640 containing 10% fetal calf serum was chilled to 4°C and used to wash the fixed cells three times. Cells were finally resuspended at appropriate concentrations in supplemented medium containing 10% human plasma.

Thus accomplished, paraformaldehyde fixation of APCs prior to addition of $HA_{306-320}$ (concentrations ranging from 0.1 to 100 μg/ml) failed to alter the response of a TLC (HA1.7), previously characterized, for example, by Eckels et al, Immunogenetics 19: 409-23 (1984), and Lamb et al, Nature 300: 66-69 (1982), HA1.7 being specific to the peptide and restricted by $DR1^+$ APCs. In contrast, enhancement of alloreactive TLC AL63.65 was completely abrogated if the replacing peptide was added after fixation, although addition of peptide four hours prior to fixation resulted in enhancement of TLC AL63.65 responses, as shown in Table 1.

TABLE 1

The observation that paraformaldehyde fixation of APCs abrogates enhancement indicates an internalization of the replacing peptide by the presenting cells. In any event, abrogation cannot be attributed to a modification of the DR1 molecule because paraformaldehyde-fixed APCs can present $HA_{306-320}$ to TLC HA1.7 with the same efficiency as do unfixed accessory cells.

Example 2. Modulation of T-cell recognition with other peptide derivatives of HA protein.

The peptides shown below in Table 2 were tested for immune-modulating activity. The experimental paradigm employed was the one described in Example 1, except that only one peptide concentration (100 μg/ml) and a single cell density (LCLs at 25 x 10^3/well) were used. Each tested peptide was a derivative of the HA protein, i.e., each peptide was obtained by modifying an isolated portion of HA, and was comprised of

(a) the following sequence:

Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr (II)

produced by deleting the first two and the last amino-acid residues from $HA_{306-320}$; or

(b) a sequence obtained by substituting several of the residues in sequence (II).

The allostimulation-responsive proliferation of TLC AL63.14 in ordinary medium was inhibited by a number of the tested HA derivatives (see Figure 2a). The T-cell clone AL63.65, which was characterized by an increased proliferative response in Example 1, displayed a qualitatively similar enhancement in the presence of several tested HA derivatives, as shown in Figure 2b.

In terms of relative ability to stimulate proliferation of DR1-restricted TLCs that are specific for HA antigen, peptide No. 1 was unusual among the tested HA-derivative peptides for being several orders of magnitude more effective than HA itself (data from

antigen-specific assay not shown). By contrast, no such large difference separated peptide No. 1 from the other tested peptides in the capacity to inhibit or enhance T-cell proliferation responsive to allostimulation.

TABLE 2

Peptide No.	Amino-Acid Sequence*
1	**Glu-Ala-Ala-Ala-**Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr
2	**Arg-**Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr
3	**Pro-**Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr
4	**Glu-Ala-**Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr
5	**Arg-**<u>Arg</u>-Tyr-Val-<u>Arg</u>-Gln-<u>Lys</u>-Thr-Leu-<u>Arg</u>-Leu-Ala-Thr
6	**Arg-**Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr
7	**Ala-**Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr

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* Highlighted residues are added to an end of sequence (II). Any residue that is substituted for another in sequence (II) is underscored.

The use of replacing peptides to modulate immune responses, in accordance with the present invention, should be effective in the particular context of modulating allergic responses, in that peptides would be identified empirically that replace allergenic peptides at the class II-molecular binding site. In other words, T-cell clones specific for allergenic peptides would be generated to use, and putative replacing peptides would be assayed, in competitive inhibition experiments as described above. Thus, any T cell-mediated immune response involving MHC-restricted peptide recognition should be modulable pursuant to the present invention.

WHAT IS CLAIMED IS:

1. A method for affecting immune responses in a mammalian subject, comprising the step of administering to said subject a replacing peptide capable of occupying an MHC binding site in lieu of an endogenous peptide, wherein said replacing peptide is administered in an amount sufficient to modulate T-cell recognition of an MHC class II molecule:endogenous peptide complex.

2. A method according to Claim 1, wherein said replacing peptide is a derivative of HA protein.

3. A method according to Claim 2, where said derivative comprises the amino-acid sequence: Cys-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly (I).

4. A method according to Claim 2, where said derivative comprises the amino-acid sequence: Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr (II).

5. A method according to Claim 4, wherein said derivative comprises an amino-acid sequence representing a one-, two- or three-residue substitution in sequence (II).

6. A pharmaceutical composition for affecting immune responses in a mammalian subject, comprising an amount of a replacing peptide capable of occupying an MHC binding site in lieu of an endogenous peptide, wherein said amount is effective to modulate T-cell recognition of an MHC class II molecule:endogenous peptide complex.

7. A pharmaceutical composition according to Claim 6, wherein said replacing peptide is a derivative of HA protein.

8. A pharmaceutical composition according to Claim 7, wherein said derivative comprises the amino-acid sequence:
Cys-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly (I).

9. A pharmaceutical composition according to Claim 7, wherein said derivative comprises the amino-acid sequence:
Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr (II).

10. A pharmaceutical composition according to Claim 9, wherein said derivative comprises an amino-acid sequence representing a one-, two- or three-residue substitution in sequence (II).

11. The use of a therapeutic amount of a replacing peptide, wherein said replacing peptide is capable of occupying an MHC binding site in lieu of an endogenous peptide, in the manufacture of a medicament for use in a method for affecting immune responses in a mammalian subject, said method being characterized by an administration to said subject of an amount of said replacing peptide sufficient to modulate T-cell recognition of an MHC class II molecule:endogenous peptide complex.

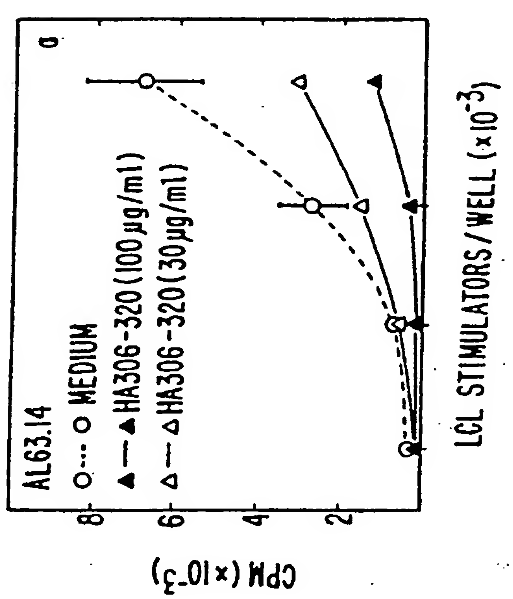


FIG. 1a

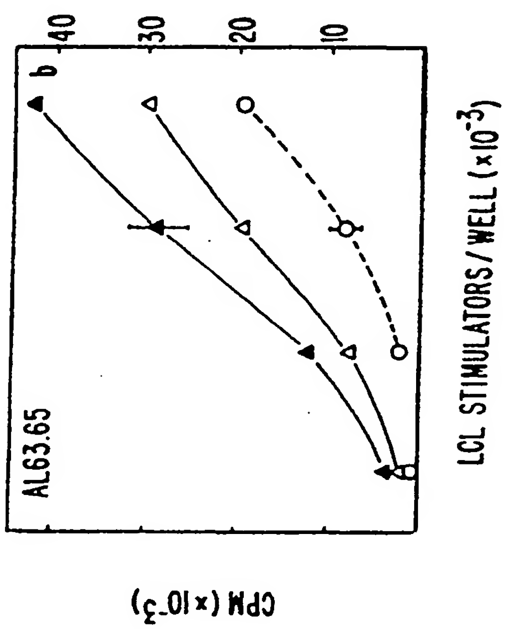


FIG. 1b

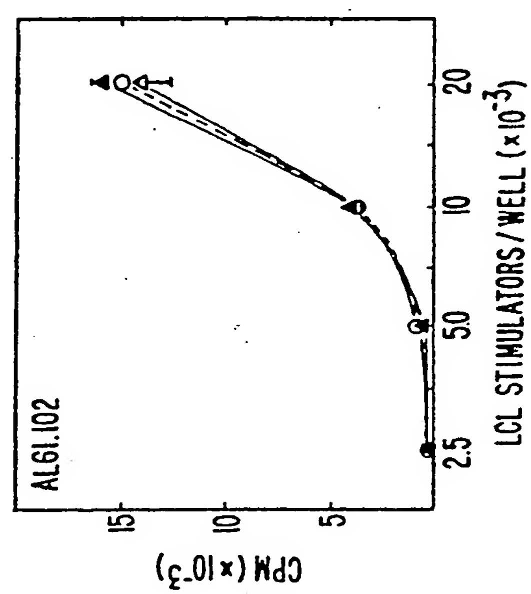


FIG. 1c

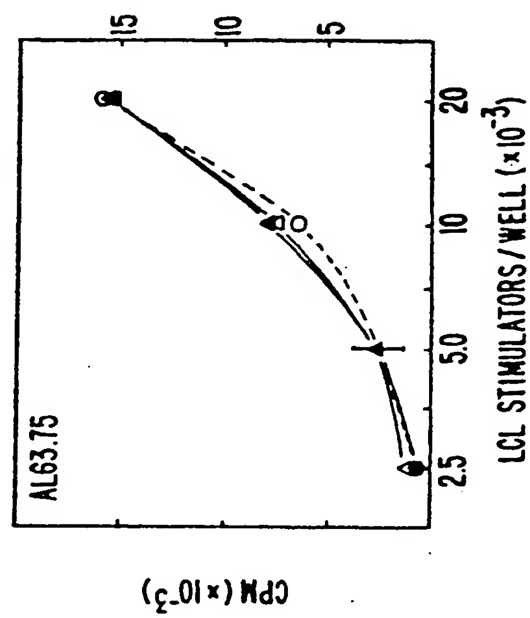


FIG. 1d

FIG. 2A

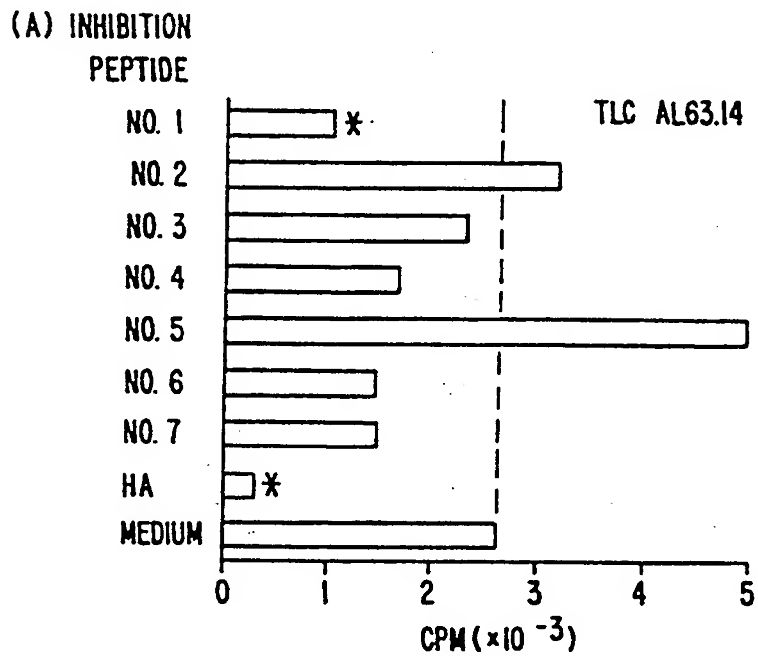
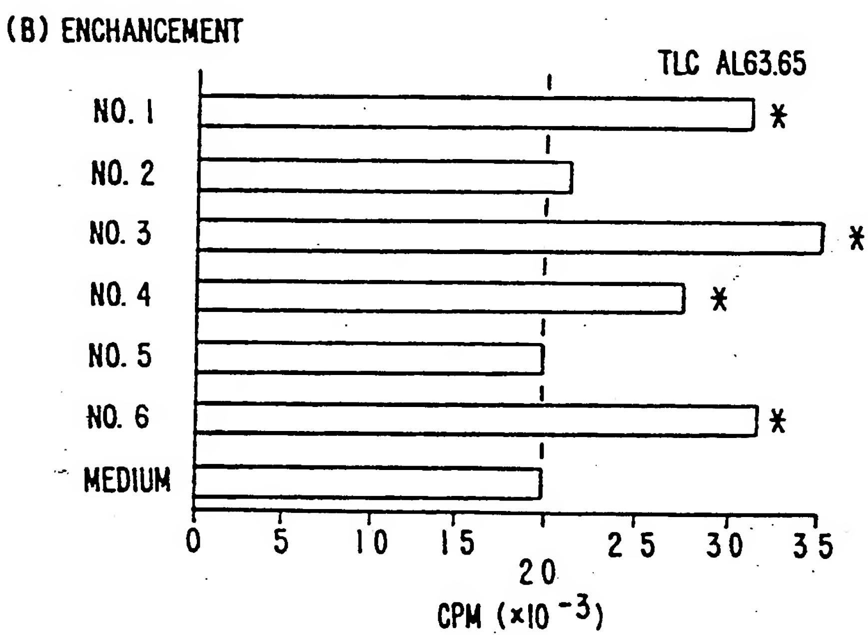


FIG. 2B



* P<0.5 (PEPTIDES NO.6 AND NO. 7 WERE JUST OUTSIDE THE 95% CONFIDENCE INTERVAL)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/00085

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: C 07 K 7/08, A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^5	C 07 K, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Proc. Natl. Acad. Sci. USA, vol. 85, November 1988, D.D. Eckels et al.: "Peptide-mediated modulation of T-cell allorecognition", pages 8191-8195, see the whole article --	1-11
P,Y	The Journal of Immunology, vol. 142, no. 5, 1 March 1989, The American Association of Immunologists, (US), C.R.A. Hewitt et al.: "Human T cell clones present antigen", pages 1429-1436 see the whole article --	1-9
P,Y	Chemical Abstracts, vol. 111, 1989, (Columbus, Ohio, US), J.B. Rothbard et al.: "Recognition of the HLA class II-peptide complex	1-9

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* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20th April 1990	25. 05. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. Peis M. PEIS

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

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